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Presence of *Pseudomonas putida* Strains Harboring Plasmids Bearing the Metallo-β-Lactamase Gene bla_{IMP} in a Hospital in Japan

Sachie Yomoda,[1] Toyoji Okubo,[2] Ayako Takahashi,[1] Masami Murakami,[1] and Shizuko Iyobe[2]*

Department of Laboratory Medicine and Clinical Laboratory Center[1] and Laboratory of Drug Resistance in Bacteria,[2] Gunma University School of Medicine, 3-39-22, Showa-machi, Maebashi 371-8511, Japan

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To determine the persistence and spread of antibiotic-resistant strains in Gunma University Hospital, 83 *Pseudomonas putida* strains (each from a different patient) were isolated from January 1997 through December 2001. Of the 83 strains isolated, 27 were resistant to carbapenems. All 27 produced metallo-β-lactamase and were found to be PCR positive for the bla_{IMP} gene. Most (22 strains) were primarily isolated from the wards (W7 [9 strains] and W4 [8 strains]). Another five bla_{IMP}-positive *P. putida* strains from wards W7 and W4 were obtained by swabbing around the water pipes. A total of 32 bla_{IMP}-positive *P. putida* strains were assessed by pulsed-field gel electrophoresis (PFGE) and testing of drug susceptibility to 10 chemotherapeutic agents. Both PFGE and MIC patterns revealed that there were long-term resident strains among inpatients and hospital environments. The bla_{IMP} genes of 22 of 32 strains were all transferable to a recipient strain of *Pseudomonas aeruginosa* by conjugation or transformation and conferred resistance to carbapenems and cephems. The bla_{IMP} plasmids were conjugally transmissible among *P. aeruginosa* strains and mediated resistance to amikacin as well as β-lactams. Ten of the 22 plasmids mediated additional resistance to gentamicin and tobramycin. Plasmids with identical DNA and drug resistance patterns were found in *P. putida* strains with identical PFGE patterns and with different PFGE patterns. We presumed that *P. putida* was one of the resident species in inpatients and especially in hospital environments, spreading drug resistance genes via plasmids among *P. putida* strains and supplying them to more pathogenically important species, such as *P. aeruginosa*.

Carbapenem-hydrolyzing metallo-β-lactamases, especially IMP-type and VIM-type metallo-β-lactamases, are clinically important, because these enzymes effectively hydrolyze almost all β-lactam antibiotics except monobactams, conferring resistance to penicillins and cephems in addition to carbapenems on pathogenic bacteria (12, 13, 18, 21, 23, 32). Since genes encoding these metallo-β-lactamases (bla_{IMP} and bla_{VIM}) and their variant genes have become easy to detect using the PCR method, dissemination of these genes in clinical isolates has been widely observed in gram-negative bacteria, especially in *Pseudomonas aeruginosa* and other non-glucose-fermenting bacteria (3, 4, 6, 8–10, 26–30, 34, 35). Recently, nosocomial infections of *Pseudomonas putida* producing VIM-type metallo-β-lactamase were reported in Italy (15). The bla_{VIM} gene was plasmid-borne but was not transferable to either *P. aeruginosa* or *Escherichia coli*. We also observed metallo-β-lactamase-producing *P. putida* strains in Gunma University Hospital, but the responsible gene was bla_{IMP}, not bla_{VIM}, and it was plasmid-borne in most of the isolates. In this paper, we report the existence of multiple-drug-resistant *P. putida* strains that harbored bla_{IMP} plasmids easily transmissible to *P. aeruginosa* in Gunma University Hospital.

MATERIALS AND METHODS

Bacterial strains. A total of 32 imipenem (IPM)-resistant *P. putida* strains isolated from 1997 to 2001 in Gunma University Hospital were investigated. The isolates consisted of 27 strains from different inpatients and 5 strains from the ward environments.

Laboratory *P. aeruginosa* strains, rifampin-resistant ML5017 (Ile$^-$, Val$^-$, Lys$^-$, Met$^-$) and rifampin-susceptible PAO4141 (Pro$^-$, Met$^-$), were used for the recipients of plasmids in conjugation or transformation (11, 18).

Media. Sensitivity test broth and sensitivity disk agar (Nissui Pharmaceutical Co., Tokyo, Japan) were used routinely. A minimum medium M9 agar (16) was used for conjugation experiments.

Antibacterial agents. The sources of antibacterial agents were as follows: imipenem and amikacin, Banyu Pharmaceutical Co., Ltd., Tokyo, Japan; meropenem, Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan; ceftazidime (CAZ), Nippon Glaxo Ltd., Tokyo, Japan; cefepime, Meiji Seika Kaisha, Ltd., Tokyo, Japan; aztreonam, Bristol-Myers Squibb K. K., Tokyo, Japan; piperacillin, Toyama Chemical Co., Ltd., Toyama, Japan; gentamicin and tobramycin, Shionogi and Co., Ltd., Osaka, Japan; norfloxacin, Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan; and rifampin, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.

Susceptibility tests. MICs were determined by an agar dilution method with sensitivity disk agar (10). Breakpoint MICs of each drug were according to National Committee for Clinical Laboratory Standards (NCCLS) standards (20).

Detection of metallo-β-lactamase-producing strains. The disk diffusion test for metallo-β-lactamase production using SMA disks (Eiken, Tokyo, Japan), which measures 2-mercaptopropionic acid inhibition of the enzyme (2), was performed. If necessary, Etest strips (AB BIODISK, Solna, Sweden), which measures the inhibitory effect of metallo-β-lactamase by EDTA (31) were used.

Detection of the metallo-β-lactamase gene bla_{IMP} by PCR. PCR amplification for detecting bla_{IMP} gene was performed as previously reported (30).

Pulsed-field gel electrophoresis (PFGE). Genomic DNAs of *P. putida* strains were prepared in agarose plugs that had been treated with lysozyme and pronase K by using Gene Path reagent kit (Bio-Rad, Tokyo, Japan) according to the manufacturer's protocol. DNA was digested with 25 U of the restriction endonuclease *Spe*I. The DNA fragments generated were then separated in a 1% agarose gel and were run in Tris-borate-EDTA buffer at 14°C on a pulsed-field apparatus (Gene Path System; Bio-Rad) at 6.0 V/cm for 19.7 h at an angle of 120°, with switching times ranging from 5.3 to 34.9 s.

Conjugation and transformation. Conjugal transfer of the bla_{IMP} gene from *P. putida* strains was performed by the membrane filter method (30) using *P. aeruginosa* ML5017 as the recipient strain. The transconjugants were selected on sensitivity disk agar containing both 8 μg of CAZ and 100 μg of rifampin per ml. The second transfer from ML5017 was performed using PAO4141 as the

* Corresponding author. Mailing address: Laboratory of Drug Resistance in Bacteria, Gunma University School of Medicine, 3-39-22, Showa-machi, Maebashi 371-8511, Japan. Phone: 81-27-220-8085. Fax: 81-27-220-8088. E-mail: siyobe@med.gunma-u.ac.jp.

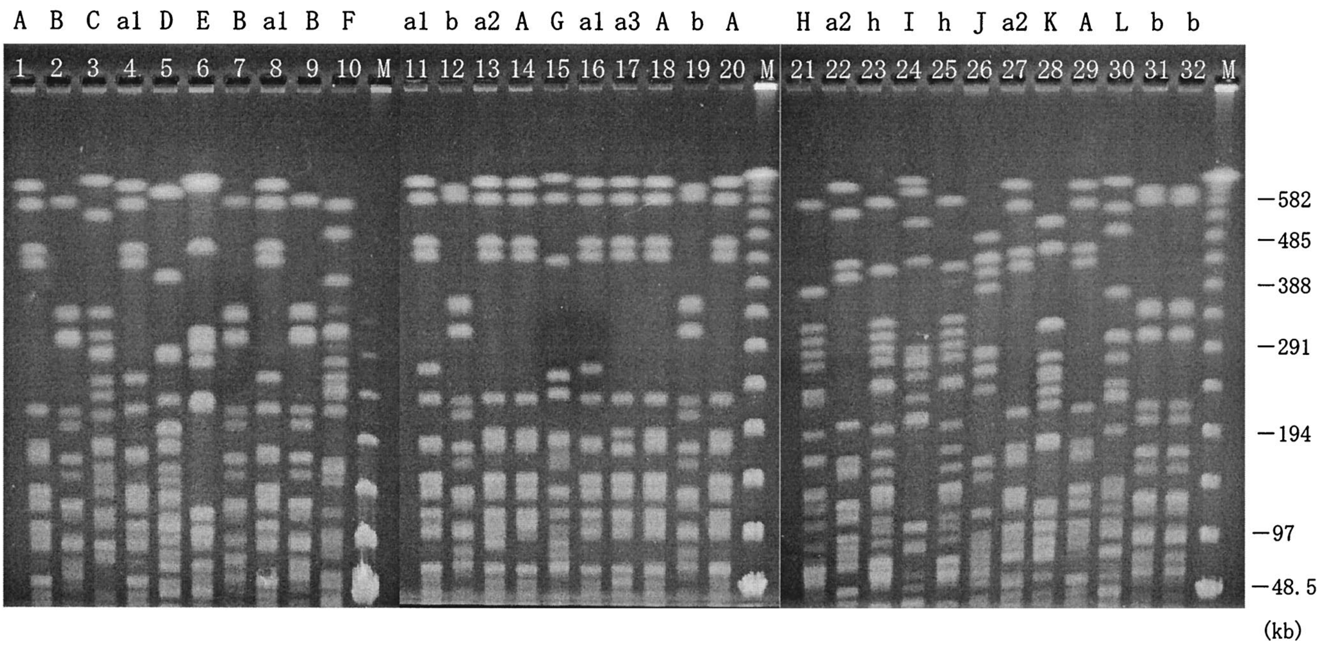

FIG. 1. PFGE patterns of *Spe*I-cut *P. putida* genomic DNAs are chronologically ordered according to isolation date, and different patterns are indicated by the letters A to L above the gel. Lowercase letters indicate patterns that are almost identical to those with the corresponding capital letter except for one discrepant segment. Numbers added to the small letters indicate different patterns. Strain numbers from 1 to 32 are shown at the top of each lane. M, size markers of DNA bands.

recipient strain, and transconjugants were selected on M9 agar containing 5 μg of proline, 5 μg of methionine, and 8 μg of CAZ per ml. Transformation experiments with plasmid DNAs from *P. putida* strains were performed by the chemical method described previously (11). Transformants of ML5017 were selected on sensitivity disk agar containing 8 μg of CAZ per ml. Transconjugants and transformants resistant to CAZ were examined for the bla_{IMP} gene by PCR.

Isolation of plasmid DNA. Plasmid DNA was isolated by alkaline sodium dodecyl sulfate method using QIAfilter Plasmid Midi kits according to the manufacturer's protocol (QIAGEN K.K., Tokyo, Japan).

RESULTS

Detection of metallo-β-lactamase-producing strains in a hospital. We isolated 83 *P. putida* strains from different inpatients of the Gunma University Hospital between January 1997 and December 2001. Twenty-seven IPM-resistant strains from patients and five IPM-resistant *P. putida* isolates from the ward environment were screened for metallo-β-lactamase production by the disk diffusion method and for the presence of bla_{IMP} gene by PCR. Metallo-β-lactamase was detected and the bla_{IMP} gene was identified in all the above-mentioned 32 IPM-resistant strains.

Isolation background and PFGE patterns of bla_{IMP}-positive *P. putida* strains. Of the 27 isolates possessing the bla_{IMP} gene, 22 were isolated from urine, and most of these were from two wards: 9 from ward W7 (internal medicine) and 8 from ward W4 (surgery). Furthermore, strains were isolated from around the water pipes of the two wards: four from ward W7 and one from ward W4.

The PFGE patterns of the genomic DNAs digested with *Spe*I endonuclease are shown in Fig. 1 in chronological order and summarized in Table 1 with the isolation date, the ward and source of the bla_{IMP}-positive *P. putida* isolates, and the underlying diseases of inpatients from whom strains were iso-

TABLE 1. *P. putida* isolates carrying the metallo-β-lactamase gene bla_{IMP}

Strain	PFGE pattern	Isolation			Underlying disease of inpatient
		Date[a]	Ward	Specimen[b]	
1	A	1997.03	W7	Urine	Prostate cancer
2	B	1997.06	W4	Urine	Gallbladder carcinoma
3	C	1997.06	W6	Urine	Pneumonia
4	a1	1997.06	S6	Urine	Prostatitis
5	D	1997.12	W3	Bile	Gallbladder polyps
6	E	1997.12	E2	Urine	Uterine cancer
7	B	1998.08	S7	Urine	Renal failure
8	a1	1998.08	S7	Urine	Anemia
9	B	1998.08	W7	Urine	Myeloma
10	F	1998.12	W4	Urine	Colon cancer
11	a1	1999.03	S4	Urine	Brain tumor
12	b	1999.06	W7	Urine	Leukemia
13	a2	1999.06	W4	Urine	Ovarian tumor
14	A	1999.10	W6	Urine	Myeloma
15	G	1999.12	W7	Urine	Lymphoma
16	a1	2000.03	W7	Urine	Leukemia
17	a3	2000.04	W7	Urine	Osteosarcoma
18	A	2000.04	W7	E	
19	b	2000.04	W7	E	
20	A	2000.04	W7	E	
21	H	2000.04	W7	E	
22	a2	2000.07	W7	Feces	Pneumonia
23	h	2000.10	W4	Urine	Hepatoma
24	I	2000.10	W4	Bile	Pancreatic cancer
25	h	2000.11	W4	Urine	Rectal cancer
26	J	2000.12	S7	Urine	Prostatic hyperplasia
27	a2	2000.12	W7	Urine	Aplastic anemia
28	K	2000.12	W4	Urine	Hepatoma
29	A	2001.08	W7	Urine	Diabetes mellitus
30	L	2001.09	W3	Bile	Hepatoma
31	b	2001.10	W4	Ascitic fluid	Hepatoma
32	b	2001.12	W4	E	

[a] Dates are shown with the year before the period and with the month after the period.
[b] E, isolate was from the ward environment.

TABLE 2. Drug susceptibilities of bla_{IMP}-positive *P. putida* strains

PFGE pattern	Strain	Isolation ward[a]	MIC (μg/ml)[b]										Transfer of bla_{IMP}[c]
			IPM	MEM	CAZ	FEP	ATM	PIP	GEN	TOB	AMK	NOR	
A	1	W7	32	>128	>128	64	32	32	64	32	32	128	−
	14	W6	64	>128	>128	128	128	64	64	64	64	128	−
	18	W7 (E)	64	>128	>128	64	32	16	64	64	64	128	−
	20	W7 (E)	64	>128	>128	64	32	32	128	128	128	128	−
	29	W7	64	>128	>128	64	64	32	128	128	64	>128	+ (TF)
a1	4	S6	64	>128	128	64	32	16	32	32	32	128	−
	8	S7	64	>128	>128	128	16	8	8	16	16	128	−
	11	S4	32	>128	128	>128	32	32	64	64	32	128	−
	16	W7	128	>128	>128	>128	32	64	128	64	128	128	−
a2	13	W4	128	>128	>128	32	64	32	64	32	>128	128	+ (C)
	22	W7	128	>128	>128	>128	64	32	128	64	>128	128	+ (C)
	27	W7	128	>128	>128	>128	64	32	128	64	>128	128	+ (C)
a3	17	W7	>128	>128	>128	>128	64	64	128	64	>128	128	+ (C)
B	2	W4	64	>128	>128	64	32	16	2	<0.5	64	1	+ (C)
	7	S7	64	>128	>128	128	32	32	2	<0.5	128	2	+ (C)
	9	W7	64	>128	>128	128	32	16	2	4	64	1	+ (TF)
b	12	W7	128	>128	>128	64	32	16	2	1	128	1	+ (C)
	19	W7 (E)	64	>128	>128	128	32	16	2	0.5	64	1	+ (C)
	31	W4	64	64	>128	128	32	16	2	0.5	128	1	+ (C)
	32	W4 (E)	64	64	>128	128	32	16	2	0.5	128	1	+ (C)
C	3	W6	16	128	64	64	32	16	2	1	64	1	+ (C)
D	5	W3	64	>128	>128	64	64	32	128	128	64	>128	+ (C)
E	6	E2	16	64	64	64	128	64	8	4	64	2	−
F	10	W4	32	>128	>128	64	32	16	8	32	64	32	+ (TF)
G	15	W7	16	>128	>128	>128	32	>128	64	64	128	4	+ (TF)
H	21	W7 (E)	64	>128	>128	128	32	16	64	32	128	1	+ (C)
h	23	W4	32	>128	>128	>128	32	16	128	64	>128	1	+ (C)
	25	W4	64	>128	>128	64	32	16	128	32	128	1	+ (C)
I	24	W4	128	>128	>128	>128	32	>128	2	8	32	16	+ (C)
J	26	S7	16	64	128	64	32	16	2	2	32	32	−
K	28	W4	8	32	32	16	8	8	2	4	32	8	+ (C)
L	30	W3	16	64	>128	>128	16	8	2	4	16	8	+ (C)

[a] E, environmental isolate.

[b] Drug abbreviations: MEM, meropenem; FEP, cefepime; ATM, aztreonam; PIP, piperacillin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; NOR, norfloxacin.

[c] Transfer of the bla_{IMP} gene was performed using a recipient of *P. aeruginosa* strain ML5017 by conjugation (C) or transformation (TF). −, no transfer.

lated. The recurrence of identical PFGE patterns observed in ward W7 suggested that these strains were long-term residents. For example, pattern A was detected in 1997 and 2001 from inpatients and in 2000 from the ward environment. Furthermore, pattern b was detected in 1999 from a patient, in 2000 from the environment in ward W7, and in 2001 from both a patient and the environment in ward W4, evidence suggesting that these strains persisted and spread to different wards.

Drug susceptibility patterns and transferability of the *bla*$_{IMP}$ gene. Table 2 shows the isolation ward, drug susceptibility, and transferability of the bla_{IMP} genes of each of the 32 *P. putida* strains grouped by PFGE patterns.

For each strain, MICs of 10 anti-*Pseudomonas* agents were determined. As defined by NCCLS standards, all 32 strains were highly or intermediately resistant to carbapenems (IPM and meropenem) and cephems (CAZ and cefepime), which are good substrates for IMP-type metallo-β-lactamase, and these strains (except strains 8 and 30) were concomitantly highly or intermediately resistant to amikacin. Furthermore, 13 of 32 strains (strains 1, 14, 18, 20, 29, 4, 11, 16, 13, 22, 27, 17, and 5) were obviously resistant to all drugs tested in this study, that is, carbapenems, cephems, the monobactam aztreonam, aminoglycosides (gentamicin, tobramycin, and amikacin), and the fluoroquinolone norfloxacin, except for the penicillin (piperacillin) whose MIC was not always high. The PFGE patterns of 11 of the 13 strains were restricted to pattern A and its relatives a1, a2, and a3, suggesting the dissemination of strains having a common clonal origin.

Some strains had exactly the same PFGE pattern, drug susceptibility pattern, and bla_{IMP} transferability. Strains 1 (isolated in 1997 from a patient) and 18 (isolated in 2000 from a ward environment) with PFGE pattern A were resistant to multiple anti-*Pseudomonas* agents and were presumed to be identical strains resident in ward W7. Isolates 22 and 27 with PFGE

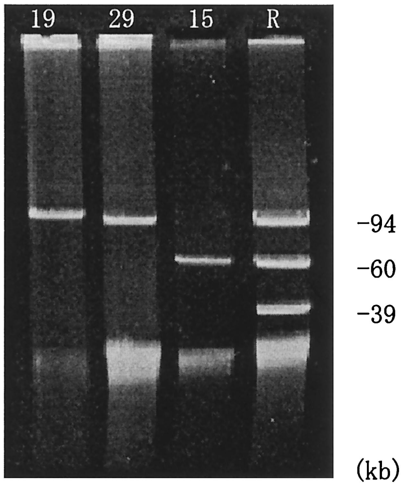

FIG. 2. Plasmid DNAs isolated from transconjugants or transformants of *P. aeruginosa* strains. The numbers at the top of each lane indicate the *P. putida* strain from which the plasmids were obtained. Lane R contains DNA from reference plasmids R100 (94 kb) (GenBank accession no. AP000342), RP4 (60 kb) (5), and Rsa (39 kb) (5).

pattern a2 were also multidrug resistant, and their bla_{IMP} genes were conjugally transmissible. They were isolated in July and December 2000 from different patients in ward W7. Strains 2 and 7 (isolated from different inpatients in different wards in 1997 and 1998, respectively) were indistinguishable in terms of their PFGE pattern (B), drug susceptibility, and presence of transmissible bla_{IMP} gene. Strains 31 and 32 (isolated in 2001 from a patient and the ward environment of W4, respectively) were also indistinguishable in PFGE pattern (b), drug susceptibility, and conjugal transmissibility of the bla_{IMP} gene.

Characterization of transferable plasmids carrying the bla_{IMP} gene. Transferability of bla_{IMP} genes of *P. putida* was first examined by conjugation, and for strains whose bla_{IMP} gene was not conjugally transferable, the gene transfer was performed by transformation with extracted DNA. The bla_{IMP} genes of 18 of 32 *P. putida* strains were transferred to *P. aeruginosa* strain ML5017 by conjugation. Plasmid DNA was isolated from 5 of the remaining 14 *P. putida* strains but was not detected in the other 9 strains. Transformation of ML5017 with the bla_{IMP} gene was successful with DNAs from four of above five strains. From transconjugants and transformants of ML5017, the bla_{IMP} gene was transferred by conjugation to a second *P. aeruginosa* recipient strain (PAO4141) at frequencies of 10^{-3} to 10^{-5} per recipient CFU, indicating that the bla_{IMP} genes were all located on the conjugative plasmids.

Agarose gel electrophoresis revealed three types of plasmids of ca. 9.8, 9.4, and 60 kb in size. Plasmid DNAs representative of 22 transconjugants or transformants of *P. aeruginosa* ML5017 are shown in Figure 2.

The plasmid size and plasmid-mediated drug resistance of *P. aeruginosa* ML5017 are shown in Table 3. Plasmids with the same size and drug resistance pattern were found to occur in strains (strains 22 and 27, strains 2 and 7, and strains 31 and 32) with the same PFGE pattern and drug susceptibility pattern.

We examined plasmid DNAs using restriction endonuclease *Eco*RI and compared digestion patterns. Figure 3 shows the

TABLE 3. Plasmid-mediated drug resistance in *P. aeruginosa*

Plasmid		MIC (μg/ml)[b]								
Source[a]	Size (kb)	IPM	MEM	CAZ	FEP	ATM	PIP	GEN	TOB	AMK
29 (A)	94	4	32	128	64	2	4	4	16	128
13 (a2)	94	4	32	128	64	2	4	<0.5	<0.5	64
22 (a2)	94	4	32	128	64	2	4	4	16	32
27 (a2)	94	4	32	128	64	2	4	4	16	64
17 (a3)	60	4	32	128	64	2	4	<0.5	<0.5	128
2 (B)	94	4	32	128	64	2	4	<0.5	<0.5	64
7 (B)	94	8	32	128	64	2	4	<0.5	<0.5	64
9 (B)	98	4	32	128	64	2	8	<0.5	<0.5	64
12 (b)	98	4	32	128	128	2	4	<0.5	<0.5	64
19 (b)	98	4	32	128	64	2	4	<0.5	<0.5	64
31 (b)	94	8	32	128	64	2	8	<0.5	<0.5	64
32 (b)	94	4	32	128	64	2	4	<0.5	<0.5	64
3 (C)	94	4	32	128	64	2	8	<0.5	<0.5	32
5 (D)	94	8	32	128	64	2	4	2	8	32
10 (F)	60	4	32	128	64	4	2	2	8	32
15 (G)	60	4	32	128	64	2	4	<0.5	<0.5	128
21 (H)	94	4	32	128	64	2	4	4	16	32
23 (h)	94	4	32	128	64	2	2	2	8	32
25 (h)	94	4	32	128	64	2	2	2	8	32
24 (I)	60	4	32	128	64	2	4	2	8	32
28 (K)	94	4	32	128	64	2	2	2	8	32
30 (L)	60	4	32	128	64	2	4	4	8	32
ML5017[c]		<0.5	<0.5	<0.5	1	2	1	<0.5	<0.5	2

[a] Strain number of *P. putida* with PFGE pattern shown in parentheses.
[b] For drug abbreviations, see Table 2, footnote *b*.
[c] Host strain of plasmid, i.e., *P. aeruginosa* ML5017.

representative *Eco*RI digestion patterns of 8 of the 15 plasmids. The ca. 98-kb plasmids from strains 12 and 19 showed identical DNA pattern and were similar to that from strain 9 with a discrepancy of a small fragment. The 94-kb plasmid DNAs from strains 29, 22, and 27 were identical, and those from strains 13, 2, 7, 31, and 32 were identical. A discrepancy of a small fragment was observed between plasmids from strains 29 and 2. It was shown that plasmids with identical DNA and drug resistance patterns were borne not only in the strains with the same PFGE pattern (a2, B, or b) but also in strains with different PFGE patterns (a2, a3, B, and b) (Table

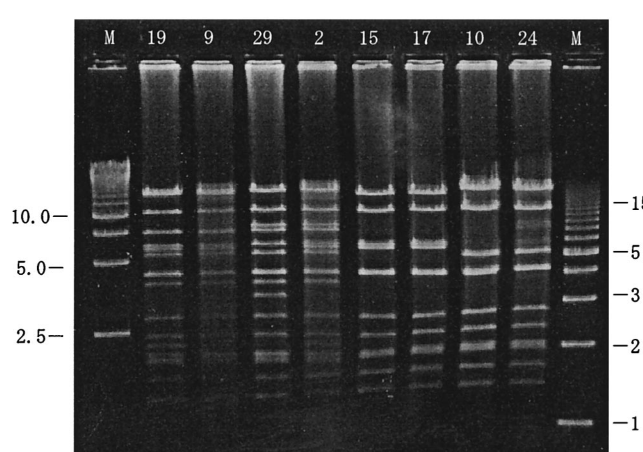

FIG. 3. Plasmid DNAs digested with restriction endonuclease *Eco*RI. The numbers above each lane are the strains from which the plasmids were obtained. M, size markers (Bio-Rad).

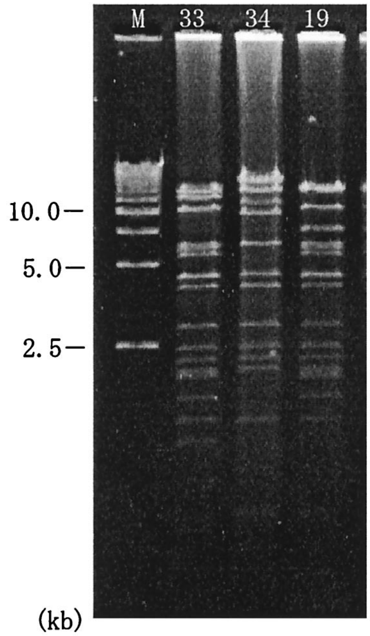

FIG. 4. *P. aeruginosa* plasmid DNAs digested with restriction endonuclease *Eco*RI. Plasmids 33 and 34 were from *P. aeruginosa* strains and plasmid 19 was from a *P. putida* strain. M, size markers (Bio-Rad).

3). The 60-kb plasmid DNAs from strains 15 and 17 and from strains 10 and 24 showed identical DNA patterns. The PFGE patterns of strains 15 and 17 were G and a3, respectively, but the DNA and drug resistance patterns of the plasmids were indistinguishable. Similarly, the PFGE patterns of strains 10 and 24 were F and I, respectively, and the plasmid DNA and drug resistance patterns were identical. Plasmids with identical sizes and drug resistance patterns were also isolated from strains showing clearly different PFGE patterns. These findings indicated that clonally identical *P. putida* strains were residing in the same ward or spreading to other wards over time.

As it seemed interesting that *P. putida* plasmids were easily transmissible by conjugation to *P. aeruginosa*, we examined bla_{IMP}-bearing plasmids from *P. aeruginosa* isolates during the same period. Two plasmids of ca. 98 kb in size were detected in *P. aeruginosa* strains which were isolated from the ward W7 environment in April 2000 (strain 33) as well as *P. putida* strain 19 and an inpatient of the ward E1 in March 1999 (strain 34). The drug resistance pattern of the plasmid from strain 33 was the same as that of *P. putida* strain 19 plasmid but that of strain 34 plasmid was different in amikacin susceptibility. The *Eco*RI-cut restriction patterns of *P. aeruginosa* plasmids are shown in Fig. 4 in comparison with that of *P. putida* strain 19 plasmid. Although *P. aeruginosa* plasmids were not identical to the *P. putida* plasmid, robust similarities were observed, especially between strain 33 and strain 19 plasmids.

DISCUSSION

P. putida, a species closely related to *P. aeruginosa*, has low pathogenic potential for most patients, with the exception of immunocompromised patients (1, 25). Over a 5-year period, *P. putida* strains resistant to carbapenems and many other kinds of chemotherapeutic agents were isolated from 27 inpatients of Gunma University Hospital. All strains of *P. putida* species that were carbapenem resistant carried the carbapenem-hydrolyzing metallo-β-lactamase gene bla_{IMP}, while 28% of 96 carbapenem-resistant strains of *P. aeruginosa* during the same period carried bla_{IMP} (data not shown). Other mechanisms which mediate carbapenem resistance were reported in *P. aeruginosa*. These mechanisms include the following: (i) deficiency of an outer membrane protein, OprD, which is a permeation route of carbapenems through the bacterial outer membrane into the periplasm where the targets of β-lactam antibiotics, penicillin-binding proteins, are located (14, 19); or (ii) efflux pump systems for antibacterial agents including carbapenems (17, 24). These mechanisms have not yet been reported in *P. putida* species. It was presumed in *P. putida* strains that mechanisms other than metallo-β-lactamase, if any, contribute to carbapenem resistance only when present in conjunction with metallo-β-lactamase production and that the bla_{IMP} gene may be essential for carbapenem resistance in this species.

Recently, multiple-drug-resistant *P. putida* isolates producing VIM-type metallo-β-lactamases were reported in Italy as a causative species of nosocomial infections (15). This also means that the metallo-β-lactamase production is an important factor for drug resistance in *P. putida* species.

Twenty-seven *P. putida* strains in Gunma University Hospital were all isolated from inpatients with underlying diseases, and 24 of 27 strains were urine and fecal isolates. Furthermore, 22 of 27 strains were isolated together with other bacterial species. These findings suggest that these *P. putida* strains are not the cause of infection but that they only colonize patients.

Since PFGE analysis of *Pseudomonas* genomes was first proposed by B. W. Holloway et al. in 1992 (7), there have been a few reports about typing in *P. putida* species. Genomic analysis using PFGE in a standard *P. putida* strain was performed (22), and it was reported that PFGE of genomic DNA was a highly discriminatory and reproducible method for epidemiological typing of clinical isolates of *P. putida* (33).

We revealed a variety of PFGE patterns among *P. putida* isolates in Gunma University Hospital and applied them to genotyping of hospital strains.

Assuming that strains with identical PFGE patterns, drug susceptibility patterns, and bla_{IMP} plasmids are identical, we could track the dissemination of the same strain within and between wards: intraward transfer (strains 22 and 27 in ward W7, strains 12 and 19 in ward W7, and strains 31 and 32 in ward W4) and interward transfer (strain 2 in ward W4 and strain 7 in specimen S7). Furthermore, the same strains were isolated from inpatients and the ward environments (strains 12 and 19 in ward W7 and strains 31 and 32 in ward W4).

These findings suggest that *P. putida* has been resident and widespread in Gunma University Hospital for a long time and that its persistence was due to adaptation to the hospital environment.

It was of interest that presumably the same plasmids (with identical phenotypes and genotypes [i.e., same drug resistance pattern and pattern of *Eco*RI-digested DNA fragments]) were identified in different strains from different hospital wards (i.e., ward W7 or W4). This suggested that plasmid dissemination by conjugal transfer was common among *P. putida* strains. We confirmed conjugal transferability of the bla_{IMP} gene of these

P. putida strains using a laboratory recipient strain of *P. putida* (data not shown).

Although pathogenicity seems not to be an important factor in *P. putida*, multiple-drug resistance cannot be disregarded, because it helps *P. putida* colonize and persist in immunocompromised inpatients, causing nosocomial infections. In addition, it was presumed that the transferable plasmids conferred resistance to multiple anti-*Pseudomonas* agents on pathogenic *P. aeruginosa* strains.

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